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THE UNIVERSITY OF ALBERTA

STUDIES ON THE TOXICITY, DISTRIBUTION AND EXCRETION OF SCANDIUM-46 IN MICE

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled: "STUDIES ON THE TOXICITY, DISTRIBUTION AND EXCRETION OF SCANDIUM-46 IN MICE", submitted by Elsayed Essam Lachine, in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The toxicity, distribution and excretion of scandium in ionic and chelated forms have been investigated. The intraperitoneal and intravenous LD_{50}'s of scandium chloride were found to be 440 mg/kg and 24 mg/kg respectively, while the LD_{50}'s for scandium-EDTA complex were 720 mg/kg and 108 mg/kg respectively in mice.

Scandium-46-chloride is largely deposited in the liver, spleen and the skeleton, excretion is slow and mostly fecal, whereas scandium-46-EDTA complex is rapidly and quantitatively excreted in the urine, a very small amount is retained in the tissues.

The biological turnover of scandium-46-EDTA complex in the body is also described. The excretion of the complex involved three compartments; a slow compartment with a biological half-life of 5351 minutes, a medium compartment having a 40 minutes biological half-life, and a fast compartment of a biological half-life of about 13 minutes.

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I. INTRODUCTION

It has been shown that upon administration of rare earth elements, they tend to be deposited preferentially in osseous tissues as well as the reticulo-endothelial system. These observations suggested that some scandium isotopes might be useful as potential scanning agents. Also, the finding that some rare earths are able to complex with nucleic acids would suggest the use of scandium in diagnosis and therapy of malignant tumors. Very few studies were undertaken on the pharmacology, toxicity, distribution and fate of scandium. In a single investigation, the LD_{50} of $ScCl_3$ was found to be 755 mg/kg and 4 g/kg for the intraperitoneal and oral routes respectively (17). Furthermore, studies on the distribution and excretion of some scandium compounds have shown that Sc-EDTA complex was almost completely excreted via the urine within 24 hours. On the other hand, Sc-citrate was retained greatly by the liver, spleen and the skeleton, excretion was slow and mostly fecal. Similar studies performed in human subjects showed that the results are consistant with those observed in mice (50, 63).

The objective of this study was to investigate the acute toxicity of $ScCl_3$ and Sc-EDTA complex. The kinetics of excretion and distribution of these compounds in various tissues of the mice were also to be noted with the ultimate aim of using short-lived scandium isotopes as potential scanning agents.

II. SURVEY OF THE LITERATURE

A. Historical

Scandium was discovered in 1879 by Nilson giving it the name "scandia" in honour of his native country. The discovery was one of the major contributions to the confirmation of Mendelev's periodic law and its tabular representation.

It was early realized that scandium salts greatly resemble in characteristics those of the heavy lanthanons. Some chemists considered scandium as a non-authentic member of the rare earths series, because ot its low atomic weight, lack of visible absorption spectra in its solutions, and the low basicity of its oxide (1).Yet, late definitions (2) of the rare earths do include scandium, and the whole group is now designated as lanthanons or lanthanides.

B. Natural Occurrence

Because the elements of this series were obtained originally as earths (oxides) from relatively rare minerals, they were characterized as rare earths. Crustal abundance data indicate clearly that the lanthanides are at least as plentiful as many of the common elements, and that the over-all supplies are potentially unlimited (3).

While the abundance of scandium in igneous rocks of the earth's crust was found to be 5 g/metric ton, which is greater than many of the lanthanides, its scatter on this planet is 0.18 atoms per 1×10^4 atoms of silicon (3). This is more abundant than yttrium, lanthanum, cerium and ytterbium (4).

It has been reported that scandium occurs in almost 800 mineral species amongst them are; silicates, meteorites, east Indian igneous rocks, coal ash, pyroxemites, ferromagnesian silicates, micas of granitic pegmatites, triplites, wiikite and bazzite (4). Some isotopes

of scandium have been found near the sites of nuclear explosions and some nuclear reactors (5,6).

C. Chemistry of scandium

The electronic configurations of scandium (atomic number 21), are almost similar to those of the heavy lanthanides (atomic number ranges from 39 to 71). Due to this similarity, they all have the same chemical properties (7). The tripositive oxidation state being a characteristic of all the rare earths. However, the dipositive and tetrapositive oxidation states were also noticed for some lanthanides (8,9, 10). It was shown that no other oxidation states of scandium occur either in solutions or in solid compounds, as the energy relationships involving the formation and hydration of gaseous scandium ions are such that the tripositive ions are stable in aquous solutions (11).

The atomic or ionic size was shown to affect those properties of the rare earth metals and their cations, respectively, that reflect attraction or lack of attraction for electrons or anions i.e. properties of basicity (7). Generally, basicity is a measure of the ease with which a species loses electrons, or of the lack of attraction which a cation has for electrons or anions (7).

Scandium has an atomic radius of 1.64 $\mathring{A}$, and an ionic or crystal radius of 0.68 $\mathring{A}$ (7), which are the smallest radii encountered within the lanthanides. This might explain the low basicity of scandium when compared to other rare earths. Also, due to the smaller size of scandium ion, it may be expected to form more highly covalent species or generally more complex species with high stability (12).

In contrast to all rare earths, scandium salts solutions show

no absorption spectra in the visible region (4), Studies performed in the infrared and the ultraviolet regions showed that these methods were not valuable in scandium analysis (13).

It was reported the preparation of a wide variety of salts including all halides except the iodide. Scandium oxalates, sulphate, oxide, hydroxide, carbonate, nitrate, acetate, borate, citrate, lactate and its complexes with polyaminocarboxylic acids were also prepared. (4)

The most notable feature in scandium determination in its pure solutions, is the lack of a specific reagent which will precipitate scandium quantitatively under a wide range of conditions (4). Gravimetric determination of scandium as the oxide or oxalate seems to be the only reproducible method, yet it has some disadvantages (14).

Scandium was shown to be 100 percent naturally monoisotopic (4), but work on induced radioactivity has demonstrated the existence of about ten isotopes of half-lives ranging from 0.87 second to 84 days. These species may be prepared by neutron bombardment of stable scandium compounds and calcium salts. Decay is by Beta and Gamma emissions yielding inactive titanium.

D. Pharmacological and Toxicological Aspects

All of the lanthanides are known to produce hypotension when administered intravenously to animals (15 - 20), and death is usually due to cardiovascular collapse coupled with respiratory paralysis. ECG records showed slow conduction time, increased height of the P-wave, decreased height of the T-wave, temporary ventricular fibrillation, and finally heart block (16). Autonomic drugs failed to counteract these fatal effects (17 - 23). Complexation of the rare earths with citrates resulted in a more pronounced drop in blood pressure, while the use of EDTA as the complexing agent did not cause such effects (24).

It has been found that all of the rare earths elements produce a high degree of irritation of the conjunctiva, but not of the cornea or iris (16). Conjunctival ulcers caused by topical applications of crystals or strong solutions of these compounds has been reported (17 - 23). These ulcers required about three weeks for healing. When the cornea was denuded, the rare earths were shown to cause an opacification which appeared after a short latent period. Although the mechanism involved is obscure, it concerns the deposition of excess calcium in the injured areas (25).

Rare earths were shown to decrease the tone and finally complete loss of contractility of the isolated ileum and uterus of rabbits, cats and dogs (16). Moreover, continuous washing did not restore the contractility of the isolated organs (17 - 23). It has then been concluded that all of these chemicals exihibit a non-specific antispasmodic effect agains acetycholine and increased intraluminal pressure. Some investigators demonstrated all of the rare earths to be cardiotoxic on isolated hearts of frogs, rabbits and cats (16, 26 - 28). In all cases, the heart stopped in diastole. Rare earths also produce a negative inotropic effect prior to the paralysis of the isolated hearts of rats, guinea pigs and rabbits (16, 29).

In an extensive study on the pharmacological effects of scandium chloride, Haley et al. (17) showed that a dosage range of 0.5 to 2mg/kg produced no observable effects in cats. On the other hand, there was a transient drop in blood pressure at a dose level of 5mg/kg, and the ECG record showed a decrease in height of the P-wave, and a return to normal size and rhythm. Complete cardiovascular collapse followed by res-

piratory paralysis was observed at a dose level of 10 mg/kg. The ECG changes included a decrease in the entire complex, an absent P-wave, an inverted T-wave, a transient ventricular fibrillation, an increased interval between the QRS complex and the T-wave, a heart block, an inverted QRS complex, and an increased T-wave. None of the effects of scandium chloride could be counteracted by atropine, and the cardiovascular collapse could not be antagonized by epinephrine.

Scandium chloride has been reported to exhibit the following actions; transient slight translucency in rabbits' cornea with loss of reaction to light by the iris (17, 25), antispasmodic effect on the isolated rabbits' ileum (17), and a decreased systole (26). It stopped the perfused frog's heart ventricle in diastole (26), had an inhibiting effect on mouse carcinoma (17), and produced an antithrombic effect but no antithromboplastic action (30).

Rare earths can be considered slightly toxic. The toxic manifestations of all these elements include ataxia, labored respiration, walking on the toes with opithotonus, writhing and sedation. Lethal effects are delayed with the maximum death rates not occuring for 2 to 4 days (16). It was noticed that there were sex differences with the females being more susceptible than males (16). In animals surviving for 30 days after administration of the element, there was a generalized peritonitis, adhesions and hemorrhagic ascitic fluid (31), a true granulomatous peritonitis, and focal hepatic necrosis (32). Once more, it has been shown that some rare earths when injected as chlorides produce fatty livers which are characterized by an abnormal accumulation of lipids in liver tissue (33, 34). The use of citrates or other chelating agents tends to obscure the lethal effects of these elements probably

by either decreasing the release of the elements or by increasing the lethability through the removal of another essential element such as calcium (16).

It was noticed that the intravenous injection of various salts of rare earths in rabbits produced liver and spleen degeneration with yellow atrophy and central lobe necrosis of the liver (17 - 23). The liver damage appeared to be sex-linked, as it was rather prominent in males than in females (17 - 23).

Inhalation of rare earths fluorides or oxides by guinea pigs resulted in progressive lung retention depending upon the duration of total exposure (35). There was also fatal delayed chemical hyperemia, and cellular eosinophilia (36), acute transient chemical pneumonitis, subacute bronchitis and bronchiolitis, focal hypertropic emphysema, and regional bronchiolar stricturing, but no granulomatosis (37).

Considering the toxic manifestations of scandium, it was reported that the symptoms of acute toxicity included immediate defecation, abdominal stretching, depressed respiration, tremors of the hind legs and sedation (17).

The intraperitoneal LD_{50} /7 days of scandium chloride was found to be 755mg/kg, while the oral LD_{50}/7 days was 4g/kg (17). Another report showed the intraperitoneal LD_{50}/28 days to be 32 mg scandium/kg (38).

E. Absorption

From the practical point of view, the most important routes of administration of any element are through the respiratory passage, the gastrointestinal tract, percutaneous injection, and through topical contamination (39).

Some investigators showed that neither stable rare earths (16), nor their radioactive isotopes (40, 41, 42) are absorbed through the gastrointestinal tract. Results revealed little absorption of rare earths following oral administration, and the recovery of a very small percentage of the administered dose (43, 44).

It was also noticed that radionuclides appearing in the air during nuclear fall out can get access to the body through the respiratory tract. The levels of radioactive elements introduced by inhalation may greatly match those injected intravenously (45 - 48).

The chemical state of the rare earth element defines the rate of its absorption from intraperitoneal, subcutaneous, and intramuscular injection sites. Readily ionizable salts such as chlorides and nitrates are slowly and incompletely absorbed, whereas the weak ionizing complexes (e.g. ethylene diamine tetra acetic acid complexes) are more rapidly and completely absorbed (49). Due to the fact that rare earths chlorides and citrates form colloidal aggregates when administered intravenously at physiological pH, it has been recommended the adjustment of rare earths solutions to a low pH to minimize the colloid formation (42, 50).

F. Metabolism and Distribution

When injected intravenously in the form of chlorides, rare earths are known to ionize and combine with carbonate, phosphate and hydroxyl ions in the blood serum to form macromolecules or colloidal aggregates (50). These macromolecules are deposited largely in the liver and to a lesser extent in the spleen after being phagocytized in the reticuloendothelial system by the macrophages (51). Further studies on the metabolism of rare earths (41, 42) revealed that the main sites of deposition

of these elements are the liver and the skeletal system. Some investigators showed that the high liver uptake of the rare earths involve the cell nuclei, mitochondria and microsomes (42). Results of Ekman et al. (52) showed that the localization of rare earths in liver and bone was due to their rapid combination with serum albumin. Others reported their capability to bind to amino acids and participate in protein formation (50, 53), protein binding (54), and nucleic acid binding (55).

The bone uptake has been accounted for as being due to cation exchange within the bone crystal itself (56), association with the organic matrix (40, 57, 58), or adsorption onto the non-growing mineralized part of the bone (59).

In an early investigation Scott et al. (60) reported that ^{46}Sc, ^{47}Sc and ^{48}Sc, when injected intravenously, had the highest concentration in the liver and reticuloendothelial system and the lowest in the skeleton. Only 25 percent of the administered dose was excreted within four days. The intramuscular injection resulted in the retention of 77 percent of the injected dose at the site of injection, with the remainder distributed in the liver, spleen, kidneys and bone.

The results of Rosoff et al. (50) showed that the distribution pattern of scandium-46, and other rare earths (^{91}Y, ^{140}La, ^{153}Sm.) in mice vary according to the ionic state of the injected element. Twenty-four hours after the intravenous injection of scandium-46-citrate, the organs with the highest uptake were the liver and spleen, while the lungs, muscles and bone were considerably low. The uptake of scandium by the liver (14%), and spleen (13%) was lower compared to other rare earths (49, 61), but in organs such as lungs (4%), kidneys (5%) and bone (6%),

scandium uptake was higher than other elements. On the other hand, following the injection of the rare earths nitriloacetate (NOA) complexes, scandium was taken up to a significantly lesser degree by the liver (7%), spleen (6), than other rare earths, while the level in the bone (5%) was comparatively high.

The results of injecting the rare earths-ethylene diamine tetraacetic acid (EDTA) complexes revealed that scandium EDTA complex is rapidly excreted, and the body retained a very small amount of the injected dose (50). Taylor (62) suggested that scandium-47 resulting from the decay of injected calcium-47 does not follow the regular metabolic pathways of scandium and up to 90 percent of the injected dose was retained by the skeleton.

Rosoff et al. (63) studied the metabolic behaviour of scandium-46 complexes with nitrilotriacetate (NTA), ethylene diamine tetraacetic acid (EDTA), and diethylenetriamine pentacetic acid (DTPA) in man. Results indicated that the weak chelate Sc-NTA leaves the vascular space very slowly. Twenty five percent of the injected dose was detected in the plasma 24 hours after injection, while with strong chelates (EDTA and DTPA) of scandium-46 were rapidly removed from the plasma. Twelve percent of the dose administered remained in the plasma after one hour past injection, and only one percent after 8 hours. Uptake studies showed the highest concentration in the spleen, then liver and vertebrae.

G. Excretion and Biological Turnover

The excretion of scandium as well as all of the rare earths elements was found to be entirely fecal (16, 41, 50). Magnusson (42) showed that fecal excretion occurs partially via the bile, and partially by

direct secretion through the intestinal mucosa.

Rosoff et al. (50) reported the presence of a great relationship between the excretion and the ionic radius of the rare earths. The smaller the ionic radius, the higher is the stability constant of the rare earth metal complex, and the greater is the excretion. For scandium, the ionic radius is 0.68 Å (7) which is small compared to the other lanthanons, and the stability constants of its chelates with EDTA and NTA are Log K_1 = 23.1 and Log K_1 = 11.4 respectively (64). The urinary excretion was found to be 100 percent for Sc-EDTA within 24 hours in mice, while for Sc-NTA excretion was only 38 percent (50). The case differs with scandium citrate, as it is readily ionizable when compared to other strong chelates, the excretion within 24 hours was 11 percent. Results showed that the excretion of the rare earths administered as chlorides was very little (50).

Human studies revealed that the main pathway of excretion of scandium and other rare earths in man is via the intestine (63, 65, 66, 67). Rosoff et al. (63) indicated that the urinary excretion of ^{46}Sc-EDTA and ^{46}Sc-DTPA was very high in 24 hours. Seventy five percent of Sc-DTPA and 64 percent of Sc-EDTA were excreted in 8 hours. The fecal excretion of these strong chelates was very low. For the weak chelate Sc-NTA, excretion was very slow and mostly fecal. The fecal excretion of ^{46}Sc-NTA was always higher than the urinary excretion.

The biological half-life of ^{46}Sc-NTA was determined by Rosoff et al. (63) in two patients and was found to be 1300 and 1557 days.

H. The Role of Chelating Agents in Radioactive Metal Mobilization

Due to the fact that some radionuclides, specially those of the

rare earth series, possess relatively long biological half-lives, much work was done on the use of complexing agents for the removal of harmful radiometals from the body. EDTA was found to be effective in the removal of radioactive yttrium and plutonium (65, 68, 69, 70). Catsch et al. (71 - 73) demonstrated the acceleration of the removal of radioactive cerium from liver, spleen and kidneys by EDTA, although it was ineffective in reducing the level of radioactive cerium in the bone. There are various reports on the removal of radioactive lanthanum (65, 66, 67), scandium (63, 65, 74), strontium (65, 75) and zinc (65, 76) using EDTA and DTPA chelates.

Results of Spencer (65) indicated the effectiveness of DTPA and EDTA in increasing the urinary excretion in man of the radioisotopes ^{140}La, ^{90}Y, ^{46}Sc, ^{65}Zn, ^{85}Sr, and ^{90}Sr. DTPA was found to be more effective than EDTA in the removal of the rare earths series and zinc-65 (65).

III. EXPERIMENTAL METHODS AND MATERIALS

A. Animal Procedures

Throughout all of this investigation, male white mice from the ALAS strain, weighing about 30 g each were used.

For the determination of the LD_{50}, six groups of animals each of 5 or 6 mice were used. The animals of each group were injected with the same dose level of the compound to be tested. Results were recorded 24 hours later, and the LD_{50} was calculated according to the method described by Litchfield and Wilcoxon (77).

For the distribution studies, animals were sacrificed by decapitation at time intervals of 5, 30, 60 and 120 minutes post injection, and the blood drained.

B. Preparation of Stable Scandium Chloride

A technical sample of Sc_2O_3 obtained from D.F. Goldsmith Chemical and Metal Corporation, Evanston, Illinois, U.S.A., was used for the preparation of the chloride. The chemical purity of Sc_2O_3 was determined gravimetrically and by neutron activation analysis prior to the preparation of $ScCl_3$.

For gravimetric analysis (14), 25 g of technical Sc_2O_3 were dissolved in concentrated HCl and heated at about 120°C until complete dissolution. Scandium was reprecipitated as scandium hydroxide by using sodium hydroxide. The gelatinous precipitate was filtered, ignited to the oxide, and weighed.

The activation analysis was performed by bombarding one gram of technical Sc_2O_3 with 14 Mev neutrons for one minute using a Cockroft-Walton Accelerator. The induced radioactivity (photopeak 1.16 Mev) was compared to that resulting from the bombardment of one gram of 99.9 percent $ScCl_3$ sample using a multichannel spectrometer and NaI(TL) crystals.

The two methods of analysis revealed that the chemical purity of technical Sc_2O_3 sample tested was 95 percent.

For the preparation of the chloride, technical Sc_2O_3 was purified by precipitation and ten grams of the product were dissolved in concentrated HCl, heated to 120°C, and the mixture evaporated down to a thick residue. After refrigeration, $ScCl_3$ separated as hydrous crystals.

C. Preparation of Non-Radioactive Sc-EDTA Complex

154.5 mg $ScCl_3$ were dissolved together with 1.489 g of the disodium salt of EDTA (a metal-to-chelate molar ratio of 1 : 4), in 6 ml distilled water. Sc-EDTA complex was then extracted with 20 ml of acetone. The extract was refrigerated and the complex separated as crystals. The mixture was filtered and the complex was air dried. Confirmation of the complexation was conducted using a 60 MC NMR instrument (Varian, model A-60D).

The spectrum of the complex was compared to that of EDTA alone. The analysis showed that, in case of the EDTA spectrum the molecule being symmetric, a broad acetic acid signal and a broad bimethylene signal were evident. On the other hand, the Sc-EDTA spectrum showed a non-symmetric molecule exhibiting a) a sharp bimethylene signal, and b) a doublet for the acetic acid.

D. Preparation of the Radioactive Scandium-46 Solution

Scandium-46 was purchased from New England Nuclear, Boston, Mass. U.S.A., in the form of $ScCl_3$ in 0.5 N HCl. The specific activity of the solution was 107mCi/mg. The radiochemical purity of the material was ascertained using a multichannel analyzer and NaI(Tl) crystals.

A portion of the original solution was diluted with distilled water so that the final activity was 10 μCi/ml. The solution for injection

was prepared by adding 15.75mg of stable $ScCl_3$ to the diluted radioactive solution.

E. Preparation of Labeled ^{46}Sc-EDTA Complex

Labeled scandium-EDTA complex was prepared using the same procedure described previously with the exception that $^{46}ScCl_3$ was substituted for the stable form.

F. Experimental Procedure

Radioactive ^{46}Sc-chloride solution (0.2ml) was slowly injected intravenously into the tail vein of the animals. Each injected dose contained 2 μCi of radioactivity and 0.45mg of stable $ScCl_3$. An exact volume of the same $^{46}ScCl_3$ solution was withdrawn, using the same syringe, at the time of injection. This was transferred to a counting vial and completed to a volume of 3ml with distilled water thus serving as a reference standard indicating the amount of activity injected into each animal. Since standards and samples were counted concurrently, it was not necessary to correct for radioactive decay.

The same procedure was followed for the uptake studies of ^{46}Sc-EDTA complex, but the dosage used was 2.5mg of the complex in 0.2ml of injected solution.

At the specified time intervals after injection, animals were sacrificed by decapitation and a blood sample was collected in a small heparinized beaker. The tissues to be investigated were excised, blotted free of blood, cleared of any adhering adipose or extraneous materials, and transferred to weighing boats to be weighed immediately.

G. Tissues Examined for the Distribution of Scandium-46

The following organs were examined for the uptake of radioactive scandium;

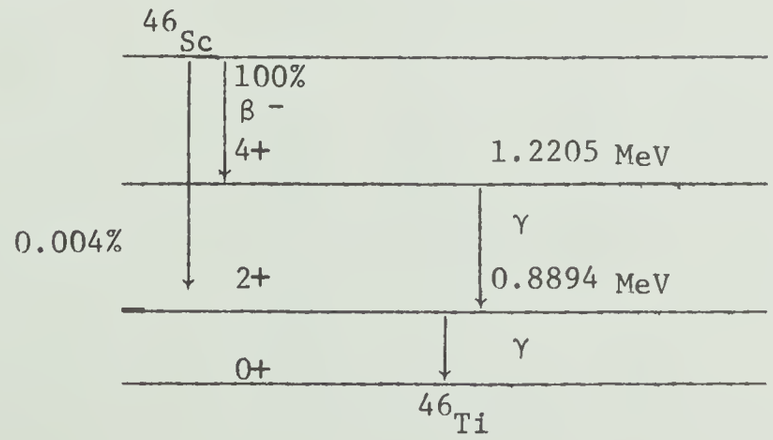
1) The whole liver
2) The whole spleen
3) The entire lungs
4) The heart
5) Both entire kidneys
6) The femur (bone)
7) Part of the hind leg muscles
8) The stomach contents
9) The whole intestine and its contents
10) The gall bladder
11) The urinary bladder
12) The whole brain
13) A blood sample.

After the samples were weighed, they were transferred to counting vials and wet ashed in concentrated nitric acid. After complete dissolution, the volume was made up to 3ml with nitric acid, and the samples were analyzed for their contents of radioactivity.

To avoid errors resulting from the adsorption of radioactive scandium on glass surfaces, all glassware used throughout this investigation was previously siliconized using the technique reported by Chase and Rabinowtz (78).

H. Analysis of Radioactivity

Scandium-46, having a half-life of 83.9 days (79) decays to stable titanium according to the following scheme (80):



The detection of gamma rays by means of a thallium-activated NaI crystal scintillation system offers a reliable means of measuring the level of scandium-46 in a sample. Tissue samples along with the previously prepared standards were each counted for 10 minutes in a Nuclear Chicago Model 4219 automatic well scintillation detector. The various samples were counted in the integral mode with the lower discrimination level adjusted to reject energies below 150Kev. All counts obtained were corrected for background radiation.

I. Whole Body Counting

Immediately after the intravenous injection of the tracer dose, each animal was placed in a plastic bottle 5 inches long and 2.2 inches wide. The necks of those bottles were cut leaving a small ridge to facilitate the removal of the animal from the counting well. To prevent the animal from escaping while counting, the upper open end of the bottles were taped. The animals were counted for one minute at 5-minutes intervals for the first hour, half hourly for the following two hours, then once daily for the next four days. Before each counting period the animals were induced to release any urine or feces. Figures 1 and 2 denote the counting arrangements and electronic circuitry used for this experiment.

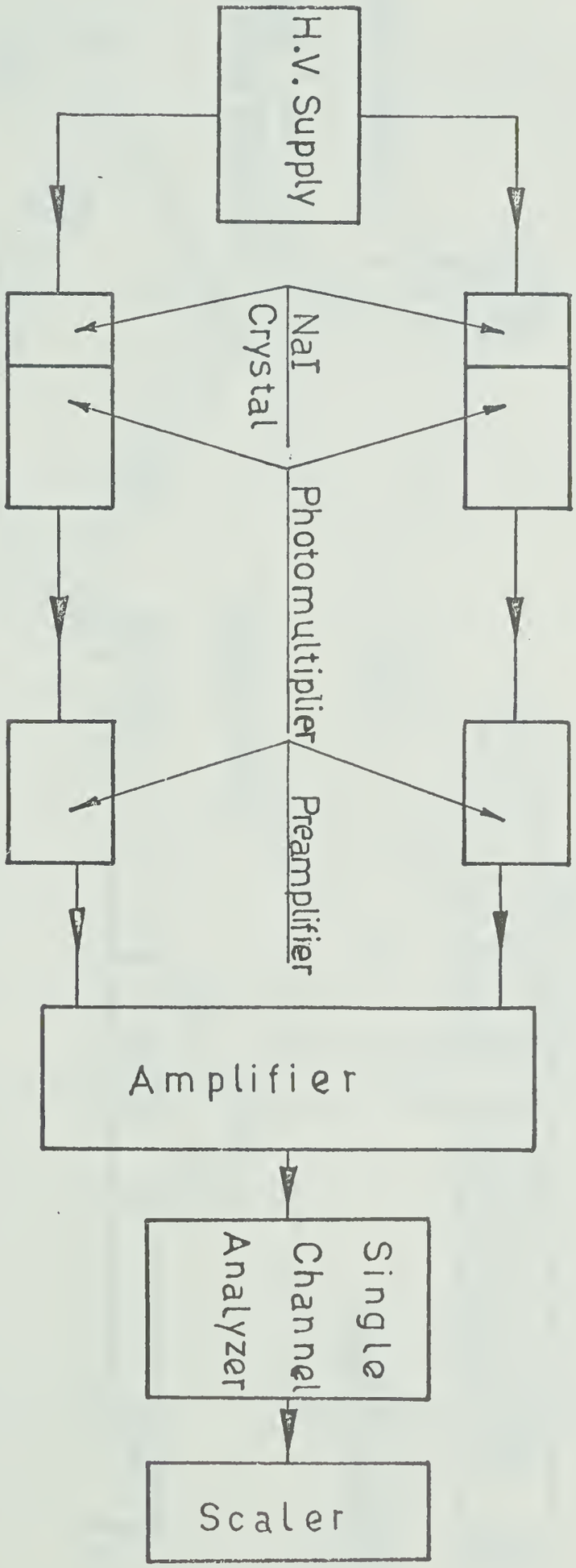


Figure 1- Electronic Circuit of the Whole Body Counter

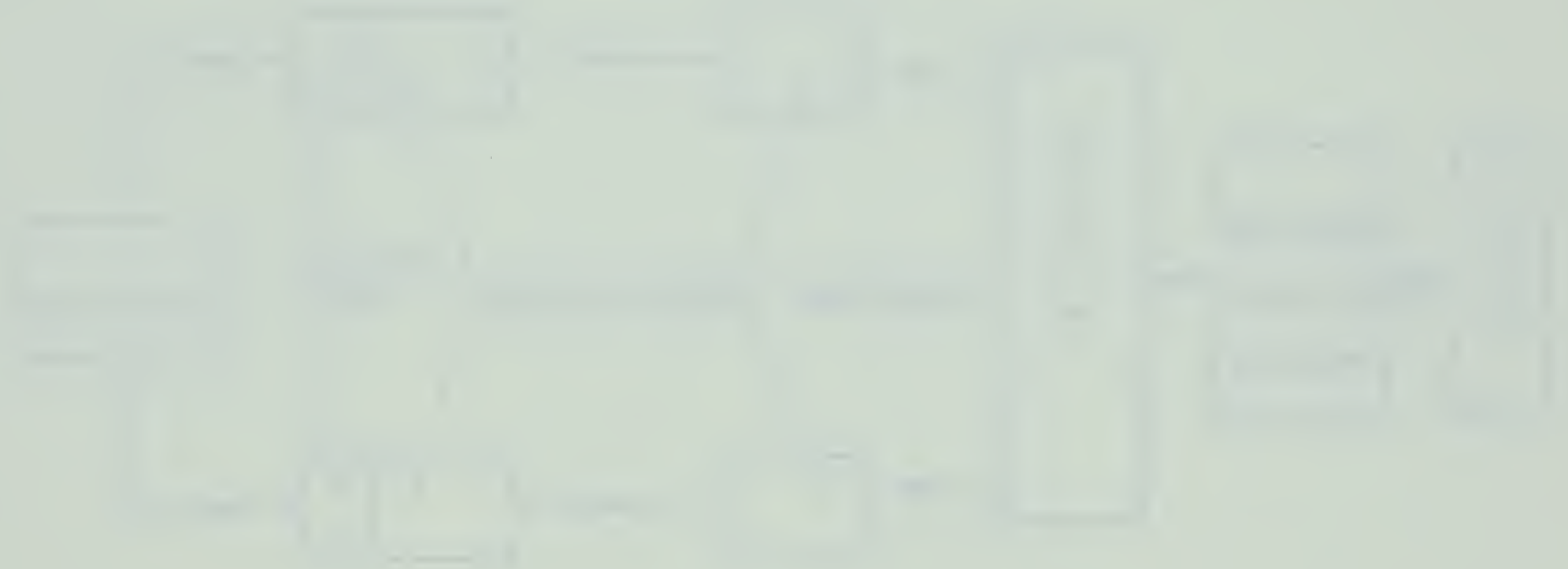
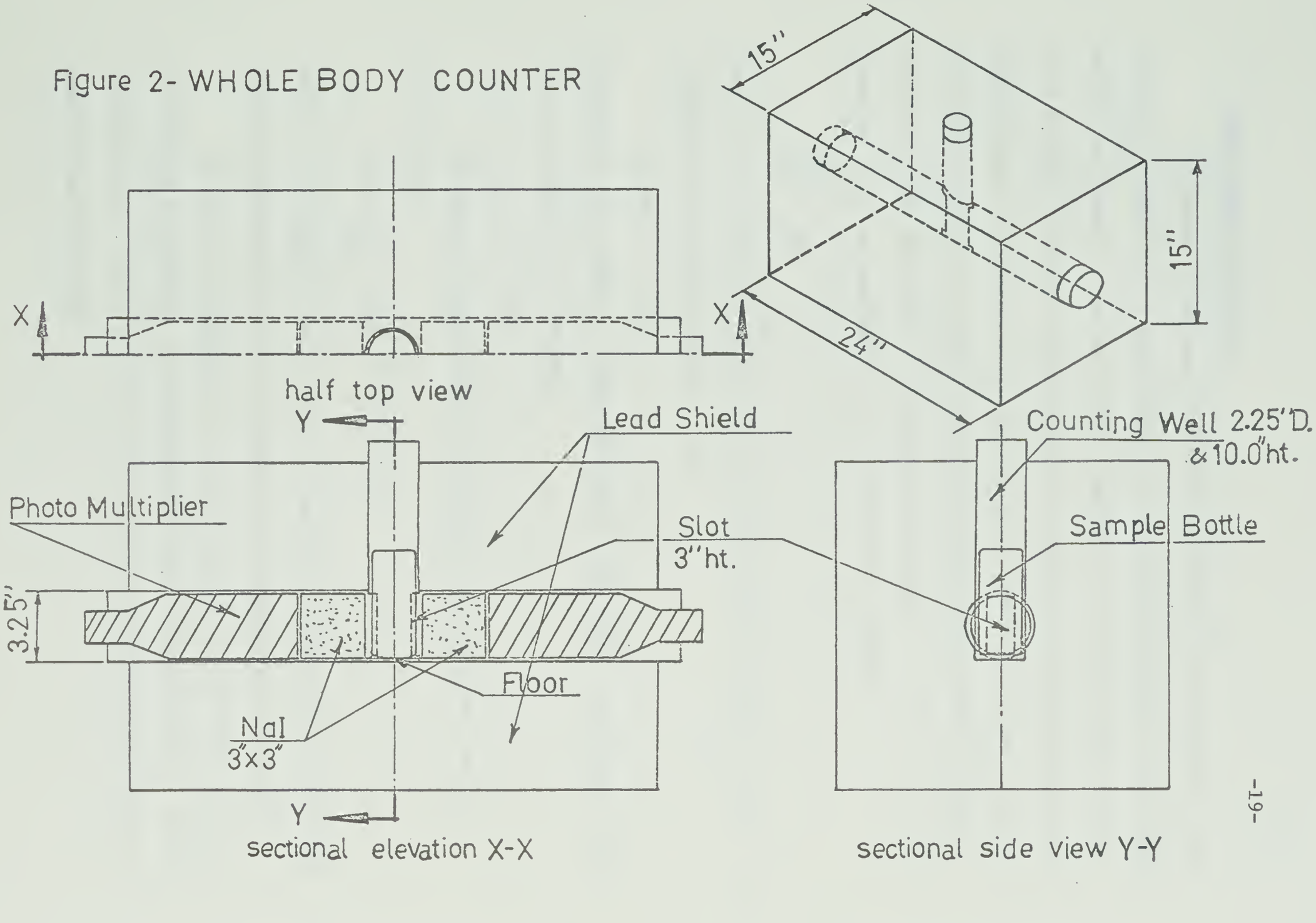


Figure 2- WHOLE BODY COUNTER



J. Compartmental Analysis

The decay of radioactivity from the body was determined by injecting the animals with a known amount of radioactive material, then determining the amount of radioactivity remaining in the body after various time periods. Using a semilog paper, the corrected counts remaining in the body were plotted as a function of time. There is a close similarity between the type of tissue desaturation curve and radioactive decay curve in which the decay of the radioactive nuclei is a first order process and could be expressed by the differential equation

$$\frac{-dN}{dt} = \lambda N$$

where λ is the decay constant, and N is the number of atoms at time t. Integration of the above equation gives

$$N = No\ e^{-\lambda t} \quad (2)$$

where No is the number of atoms present at time zero. In terms of count rate, A, equation (2) becomes,

$$A = Ao\ e^{-\lambda t} \quad (3)$$

If several decay species are present, the observed count rate f (t) is then

$$f(t) = \sum_{i=1}^{n} (Ao)_i\ e^{-\lambda it} \quad (4)$$

where i are integers from 1 to n. It should be noted that in this equation the exponentials are all assumed to be separate and unrelated.

In order to determine if the last portion of the curve consisted of a single exponential or an exponential plus a bound fraction, the method described by Dick and Lee (81) was used. The latter technique is based on the following:

Supposing that a bound fraction exists then,

$$f(t) = A\, e^{-\lambda t} + B$$

and since

$$\frac{d\, f(t)}{d(t)} = -\lambda\, A\, e^{-\lambda t}$$

then

$$\frac{d\, f(t)}{d\, t} = -\lambda[f(t) - B]$$

$$= \lambda B - \lambda f(t)$$

If the relationship between $\frac{d\, f(t)}{d\, t}$ (cpm/m) and f(t) is expressed on a normal graph a straight line is obtained whose X intercept is B (i.e. the bound fraction). The reason is that when $\frac{d\, f(t)}{d\, t} = 0$, then B = f(t), and f(t) = B.

On the other hand, if no bound fraction is present, in this case when $\frac{d\, f(t)}{d\, t} = 0$, f(t) = 0, and the straight line is expected to pass through the origin. The number of compartments is then determined by simple curve peeling techniques using either a graphical method or a Computer program after subtraction of the bound fraction.

The Computer analysis program used in this investigation was based on weighed regression. The slope of the last three points of the graph is determined. This is followed by the slope calculation of the same line after the addition of the data from a fourth point. The standard deviation of the best fit is computed, and the t value for the corresponding degrees of freedom is calculated. This procedure is repeated with additional points until the slope changes significantly. The computer is then allowed to strip the latter part of the curve with a calculation of the intercept and the half-life.

IV. RESULTS

A. Toxicity Studies

In order to evaluate the proper dose to be administered to the animals an acute toxicity study was done. The LD_{50} determination of $ScCl_3$ and Sc-EDTA complex were performed on mice using two routes of administration, namely the intraperitoneal and the intravenous methods. Results were recorded 24 hours after injection and the evaluation of the LD_{50} was achieved using the method described by Litchfield and Wilcoxon (77). In addition to its simplicity and time saving this method offers the following:

1) It determines the LD_{50} with its 95 percent confidence limits.
2) It recognizes heterogeneity of data when present by using the statistical parameter $(Chi)^2$, and
3) It uses zero and 100 percent observations to their best effect.

The results of this study are given in tables, I, II, III and IV.

TABLE I

Determination of the intraperitoneal LD_{50} of non-radioactive $ScCl_3$

Dose mg/kg	Dead/tested	Observed % dead	Expected % dead	Observed-expected	Contribution to $(Chi)^2$
150	0/6	0(2.9)	9	6.1	0.048
300	2/6	33	33	0.0	0.0
600	4/6	66	67	1.0	0.0008
900	5/6	83	83	0.0	0.0
1200	6/6	100(96.8)	90.5	6.3	0.05
1800	6/6	100(99.0)	97	2.0	0.014

Total animals = 36 Total = 0.0128

Number of doses, K = 6 $(Chi)^2$ = 0.0128 X 6 = 0.6768

Animal/dose - 36/6 = 6 Degrees of freedom, n = K - 2 = 4

$(Chi)^2$ was obtained from tables for n of 4 - 9.49, since 0.6768 is less than 9.49, therefore the data are not significantly heterogenous.

LD_{16} - 195mg/kg

LD_{50} = 440mg/kg

LD_{84} = 920mg/kg

Slope function, $S = \frac{LD_{84}/LD_{50} + LD_{50}/LD_{16}}{2} = \frac{920/440 + 440/195}{2} = 2.17$

The number of animals whose expected effect lies between 16 and 84 percent, N' = 18

Factor for LD_{50}, $f_{LD_{50}} = (S)^{2.77\sqrt{N'}} = (2.17)^{2.77\sqrt{18}} = 1.65$

Then, the lower limit = 440/1.65 = 266mg/kg

and, the upper limit = 440 X 1.65 = 727mg/kg

From the above data, the intraperitoneal LD_{50} of $ScCl_3$ with 95 percent confidence limits was calculated to be 440 (266 to 726)mg/kg.

TABLE II

Determination of the intravenous LD_{50} of non-radioactive $ScCl_3$

Dose mg/kg	Dead/tested	Observed % dead	Expected % dead	Observed-expected	Contribution to $(Chi)^2$
22.2	0/6	0(3.8)	12	8.2	0.06
23.4	2/6	33	33	0.0	0.0
24.6	4/6	66	62	4.0	0.006
25.8	5/6	83	82	1.0	0.001
27.0	6/6	100(98.4)	95	3.4	0.024
28.2	6/6	100(99.5)	98.2	1.3	0.010

Total animals = 36 Total = 0.101

Number of doses, K = 6 $(Chi)^2 = 0.101 \times 6 = 0.606$

Animals/dose = 36/6 = 6 Degrees of freedom, n = K-2 = 4

$(Chi)^2$ was obtained from tables for n of 4 = 9.49, since 0.606 is less than 9.49, therefore, the data are not significantly heterogenous.

LD_{16} = 22.5mg/kg

LD_{50} = 24.0mg/kg

LD_{84} = 26.0mg/kg

Slope function, $S = \frac{LD_{84}/LD_{50} + LD_{50}/LD_{16}}{2} = \frac{26/24 + 24/22.5}{2} = 107$

The number of animals whose expected effect lies between 16 and 84 percent, N' = 18

Factor for LD_{50}, for $f_{LD_{50}} = (S)^{2.77\sqrt{N'}} = (1.07)^{2.77\sqrt{18}} = 1.06$

Then, the lower limit = 24/1.06 = 22.6mg/kg

and, the upper limit = 24 X 1.06 = 25.4mg/kg

From the above data, the intravenous LD_{50} of $ScCl_3$ with 95 percent confidence limits was calculated to be 24 (22.6 to 25.4)mg/kg.

TABLE III

Determination of the intraperitoneal LD_{50} of non-radioactive Sc-EDTA complex

Dose mg/kg	Dead/tested	Observed % dead	Expected % dead	Observed-expected	Contribution to $(Chi)^2$
633.3	0/5	0(3.8)	12	8.2	0.060
666.7	1/5	20	27	7.0	0.035
700.0	2/5	40	42	2.0	0.0016
733.3	3/5	60	58	2.0	0.0016
766.7	4/5	80	75	5.0	0.0125
800.0	5/5	100(95.7)	84	11.1	0.1000
833.3	5/5	100(97.4)	92	5.4	0.0400

Total animals = 35 Total = 0.2507

Number of doses, K = 7 $(Chi)^2 = 0.2507 \times 5 = 1.2535$

Animals/dose = 35/7 = 5 Degrees of freedom, n = K - 2 = 5

$(Chi)^2$ was obtained from tables for n of 5 = 11.1, since 1.2535 is less than 11.1, therefore, the data are not significantly heterogenous.

LD_{16} = 640mg/kg

LD_{50} = 720mg/kg

LD_{84} = 800mg/kg

$$\text{Slope function, } S = \frac{LD_{84}/LD_{50} + LD_{50}/LD_{16}}{2} = \frac{800/720 + 720/640}{2} = 1.115$$

The number of animals whose expected effect lies between 16 and 84 percent, N' = 25

$$\text{Factor for } LD_{50},\ f_{LD_{50}} = (S)^{2.77\sqrt{N'}} = (1.115)^{2.77\sqrt{2S}} = 1.08$$

Then, the lower limit - 720/1.08 = 667mg/kg

and, the upper limit = 720 X 1.08 = 778mg/kg

From the above data, the intraperitoneal LD_{50} of Sc-EDTA complex with 95 percent confidence limits was calculated to be 720 (667 to 778) mg/kg.

TABLE IV

Determination of the intravenous LD_{50} of non-radioactive Sc-EDTA complex

Dose mg/kg	Dead/tested	Observed % dead	Expected % dead	Observed-expected	Contribution to $(Chi)^2$
100.0	0/5	0(3.2)	10	6.8	0.05
103.3	1/5	20	20	0.0	0.00
106.7	2/5	40	42	2.0	0.002
110.0	3/5	60	64	4.0	0.007
113.3	4/5	80	82	2.0	0.003
116.7	5/5	100(98.0)	94	4.0	0.002

Total of animals = 30 Total = 0.064

Number of doses, K = 6 $(Chi)^2$ = 0.064 X 5 = 0.320

Animals/dose = 30/6 = 5 Degrees of freedom, n = K -2 = 4

$(Chi)^2$ was obtained from tables for n of 4 = 9.49, since 0.320 is less than 9.49 therefore, the data are not sifnificantly heterogenous.

LD_{16} = 101mg/kg

LD_{50} = 108mg/kg

LD_{84} = 115mg/kg

$$\text{Slope function, } S = \frac{LD_{84}/LD_{50} + LD_{50}/LD_{16}}{2} = \frac{115/108 + 108/101}{2} = 1.015$$

The number of animals whose expected effect lies between 16 and 84 percent, N' = 25.

$$\text{Factor for } LD_{50},\ f_{LD_{50}} = (S)^{2.77\ \sqrt{N'}} = (1.015)^{2.77\ \sqrt{25}} = 1.06$$

Then, the lower limit = 108/1.06 = 102mg/kg

and, the upper limit = 108 X 1.06 = 114mg/kg

From the above data, the intravenous LD_{50} of Sc-EDTA with 95 percent confidence limits was calculated to be 108 (102 to 114)mg/kg.

B. Time Distribution Studies of Radioactive Scandium Chloride ($^{46}ScCl_3$)

The results of tissue distribution of scandium chloride in mice are given in tables V, VI, VII and VIII.

The most striking feature of $^{46}ScCl_3$ was its high uptake by the liver, spleen,bone and muscles. Almost 18 percent of the administered dose of radio-scandium was concentrated in the liver 5 minutes after injection. The level of deposited scandium increased to 33 percent after 2 hours (Fig. 3).

The next highest concentration was found to be in the skeletal muscles. Whereas 8.6 percent of the injected $^{46}ScCl_3$ was deposited within 5 minutes after administration, rising slightly to 9.2 percent after 30 minutes, it finally decreased to 8 percent after 2 hours (Fig. 4).

The level of radioactivity in the skeletal system was found to be 6.2 percent of the injected dose after 5 minutes, decreasing to 4.5 percent after 30 minutes, then increasing again to 6.7 percent at the end of two hours (Fig. 5).

The initial concentration in the lungs was 6 percent within 5 minutes after intravenous administration. This level gradually decreased to 3.7 percent after a period of 2 hours (Fig. 6).

On the other hand, the concentration in the spleen increased from 1 percent after 5 minutes post-injection to 2.4 percent after 2 hours (Fig. 7).

The kidneys showed a constant uptake figure of 2.5 percent throughout the time intervals of this experiment (Fig. 8).

An important observation was that the blood level of $^{46}ScCl_3$ remained high throughout the two hours. It was found to be about 55 per-

TABLE V*

Tissue distribution of $^{46}ScCl_3$ five minutes after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	7	18.00 ± 0.98	13.86 ± 1.52
spleen	7	1.04 ± 0.06	13.41 ± 2.27
lungs	7	6.04 ± 0.56	28.08 ± 2.88
heart	7	0.66 ± 0.04	7.48 ± 0.76
kidneys	7	2.27 ± 0.11	5.48 ± 0.75
bone	7	6.17 ± 0.96	3.02 ± 0.41
muscles	7	8.58 ± 1.48	0.64 ± 0.11
stomach contents	6	0.44 ± 0.06	0.81 ± 0.28
intestine and its contents	7	2.68 ± 0.24	1.04 ± 0.13
gall bladder	6	0.05 ± 0.01	8.89 ± 0.52
brain	7	0.38 ± 0.04	1.18 ± 0.11
blood	6	55.41 ± 4.23	24.10 ± 1.83[d]
urinary bladder and its contents	6	0.12 ± 0.02	0.69 ± 0.10

* for the legend to this table see page 33

LEGEND for TABLES V to XII

a. Total bone and muscles were calculated on the basis of 6% and 45% respectively of the body weight of the mouse (82).

b. Total blood was calculated on the basis of 2.3ml/30g mouse (83).

c. Mean $\pm$ S.E.

d. Expressed as percent/ml.

TABLE VI*

Tissue distribution of $^{46}ScCl_3$ thirty minutes after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	8	20.67 ± 2.15	15.28 ± 1.86
spleen	7	1.84 ± 0.15	17.94 ± 2.04
lungs	7	4.30 ± 0.52	21.55 ± 2.41
heart	7	0.73 ± 0.07	6.75 ± 0.84
kidneys	7	2.76 ± 0.22	6.38 ± 1.40
bone	8	4.50 ± 0.50	2.51 ± 0.33
muscles	8	9.21 ± 0.68	0.62 ± 0.03
stomach contents	7	0.53 ± 0.11	2.36 ± 0.63
intestine and its contents	7	3.05 ± 0.34	1.63 ± 0.20
gall bladder	7	0.02 ± 0.00	3.38 ± 0.80
brain	7	0.23 ± 0.02	0.85 ± 0.13
blood	6	49.82 ± 2.19	21.66 ± 0.95[d]
urinary bladder and its contents	9	0.13 ± 0.03	0.78 ± 0.05

* for the legend to this table see page 33

TABLE VII*

Tissue distribution of $^{46}ScCl_3$ one hour after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	7	30.20 ± 1.55	18.27 ± 1.38
spleen	7	2.39 ± 0.31	14.52 ± 2.90
lungs	7	4.02 ± 0.68	14.57 ± 2.00
heart	7	0.61 ± 0.07	4.34 ± 0.29
kidneys	7	2.42 ± 0.19	4.57 ± 0.48
bone	9	4.41 ± 0.29	2.41 ± 0.11
muscles	9	8.03 ± 0.58	0.61 ± 0.04
stomach contents	6	0.09 ± 0.02	0.72 ± 0.21
intestine and its contents	6	1.35 ± 0.04	1.19 ± 0.09
gall bladder	6	0.02 ± 0.00	4.18 ± 0.86
brain	6	0.66 ± 0.09	0.85 ± 0.08
blood	6	49.42 ± 2.93	21.49 ± 1.27[d]
urinary bladder and its contents	7	0.21 ± 0.01	0.61 ± 0.03

* for the legend to this table see page 33

TABLE VIII*

Tissue distribution of $^{46}ScCl_3$ two hours after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	7	33.68 ± 0.95	19.21 ± 1.36
spleen	7	2.04 ± 0.40	17.88 ± 4.24
lungs	7	3.71 ± 0.35	13.22 ± 2.06
heart	6	0.66 ± 0.12	4.02 ± 0.58
kidneys	6	2.48 ± 0.20	3.88 ± 0.40
bone	6	6.71 ± 0.88	3.75 ± 0.48
muscles	6	8.52 ± 1.31	0.52 ± 0.09
stomach contents	6	0.26 ± 0.04	0.82 ± 0.24
intestine and its contents	6	3.87 ± 0.51	1.44 ± 0.18
gall bladder	6	0.02 ± 0.00	5.65 ± 0.67
brain	6	0.16 ± 0.02	0.36 ± 0.05
blood	6	36.22 ± 1.34	15.75 ± 0.60[d]
urinary bladder and its contents	6	0.13 ± 0.02	0.48 ± 0.08

* for the legend to this table see page 33

cent of the administered dose 5 minutes after injection, the level stayed at 50 percent for one hour, but after 2 hours had elapsed it was only 36 percent of the injected dose (Fig. 9). It was noticed that the decline in radio-scandium chloride blood level was paralled by an increased deposition in the liver, bone and spleen, and a decreased uptake by the lungs.

Other organs such as the heart, brain and stomach contents were shown not to contribute much to the deposited $^{46}ScCl_3$.

The amount of radioactive $ScCl_3$ in the muscles of the urinary bladder and its contents never exceeded 0.2 percent of the administered dose, denoting that the excretion via the urine was minimal. On the other hand, the intestine and its contents were found to concentrate about 2.7 percent of the injected dose 5 minutes after administration, increasing to about 3 percent 30 minutes later. After one hour, the level dropped to 1.4 percent, but finally after 2 hours it concentrated almost 4 percent. These levels are relatively high as compared to urinary bladder and its contents. These results may also indicate that the excretion of $^{46}ScCl_3$ via feces is most likely.

C. Time Distribution Studies of Radioactive Scandium EDTA Complex (^{46}Sc-EDTA).

Results of tissue distribution of scandium-EDTA complex are given in tables IX, X, XI and XII. These results revealed a definite pattern for the distribution and uptake of radioactive scandium-EDTA complex, that is a minimal deposition in tissues and a rapid and quantitative excretion through the urine.

Five minutes after injection, the skeletal muscles concentrated

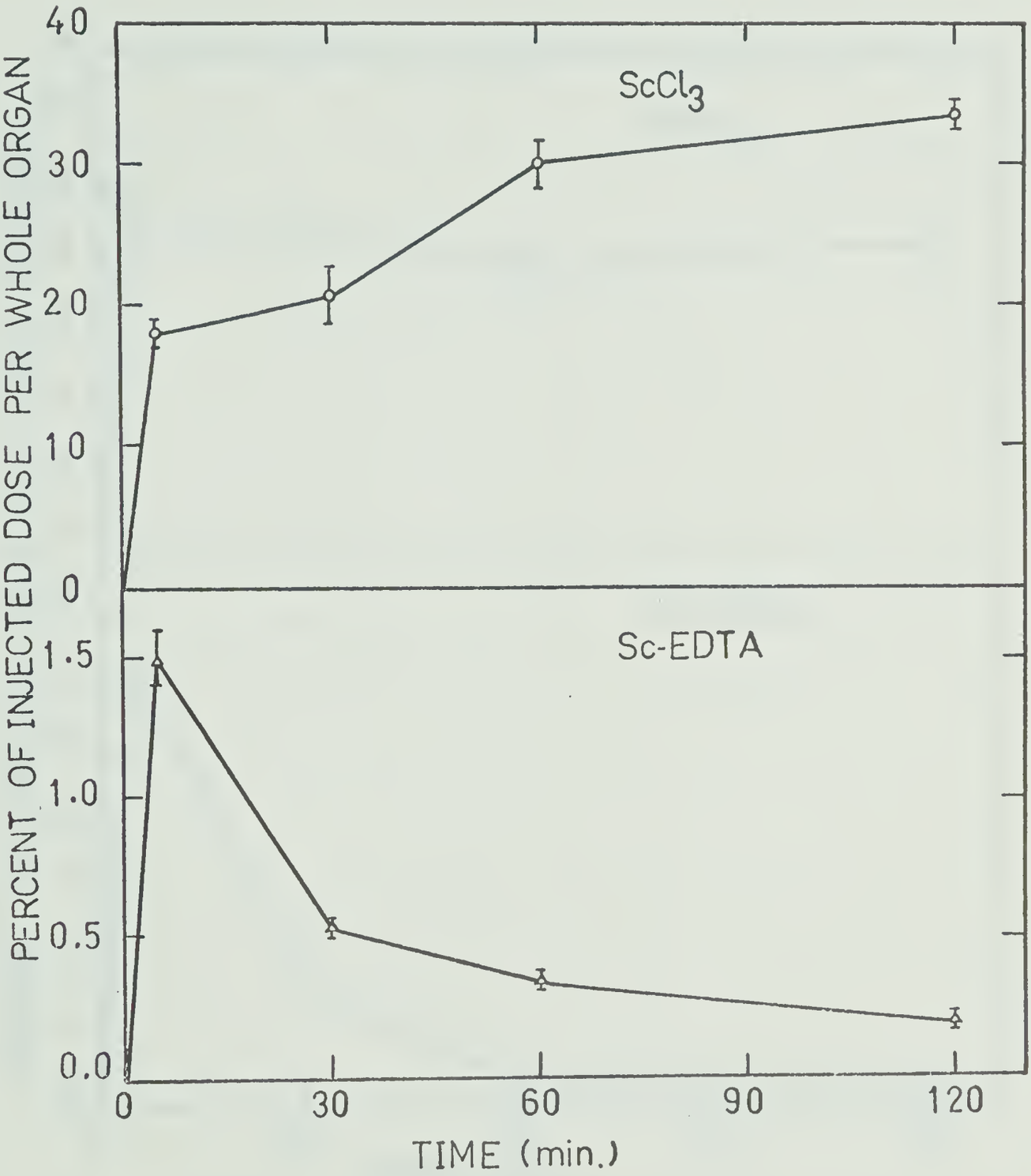


Figure 3. Uptake of scandium-46 by the mouse liver after intravenous injection

Vertical bars I represent the standard error of the mean.

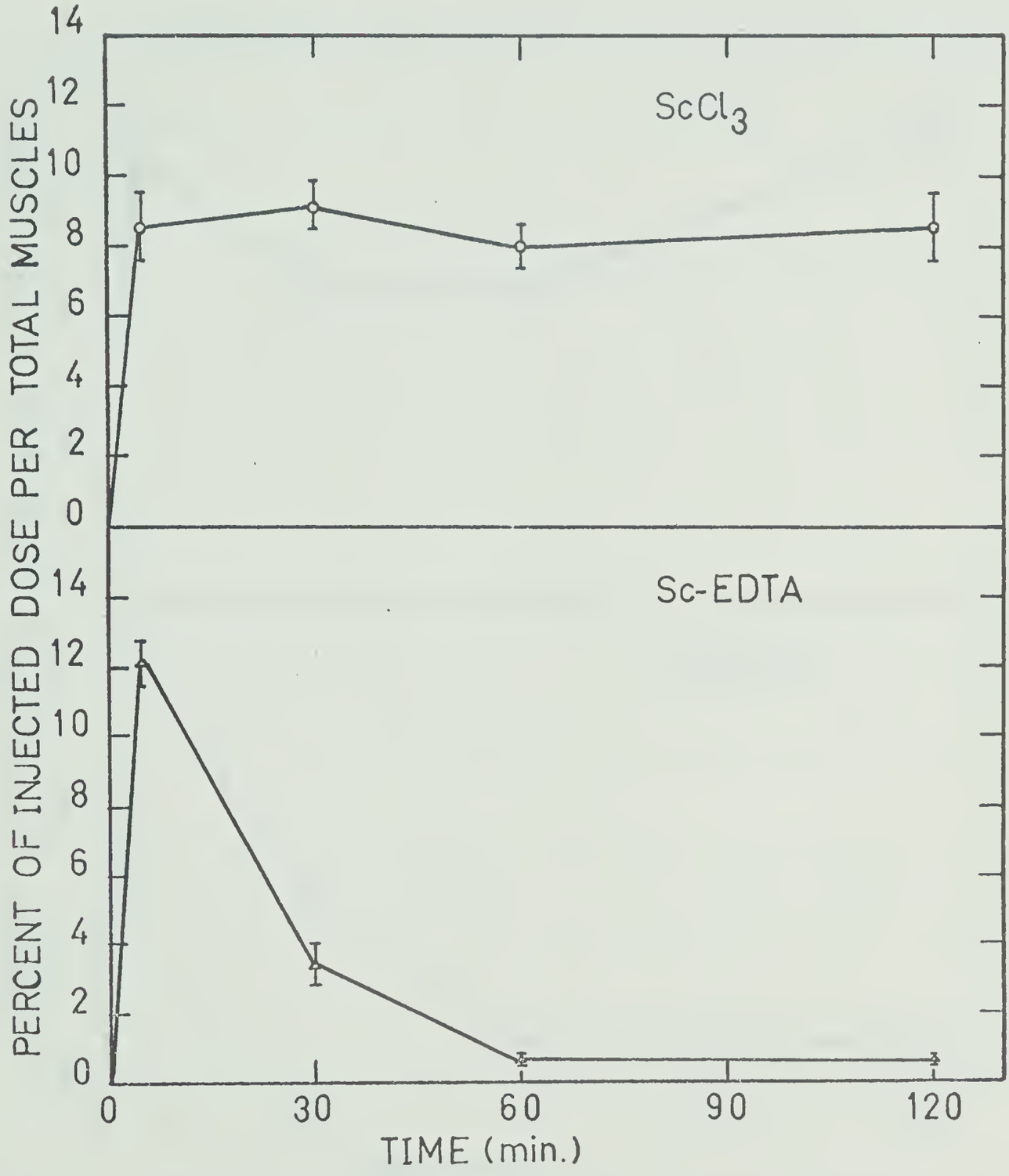


Figure 4. Uptake of scandium-46 by the mouse total muscles after intravenous injection

Vertical bars I represent the standard error of the mean.

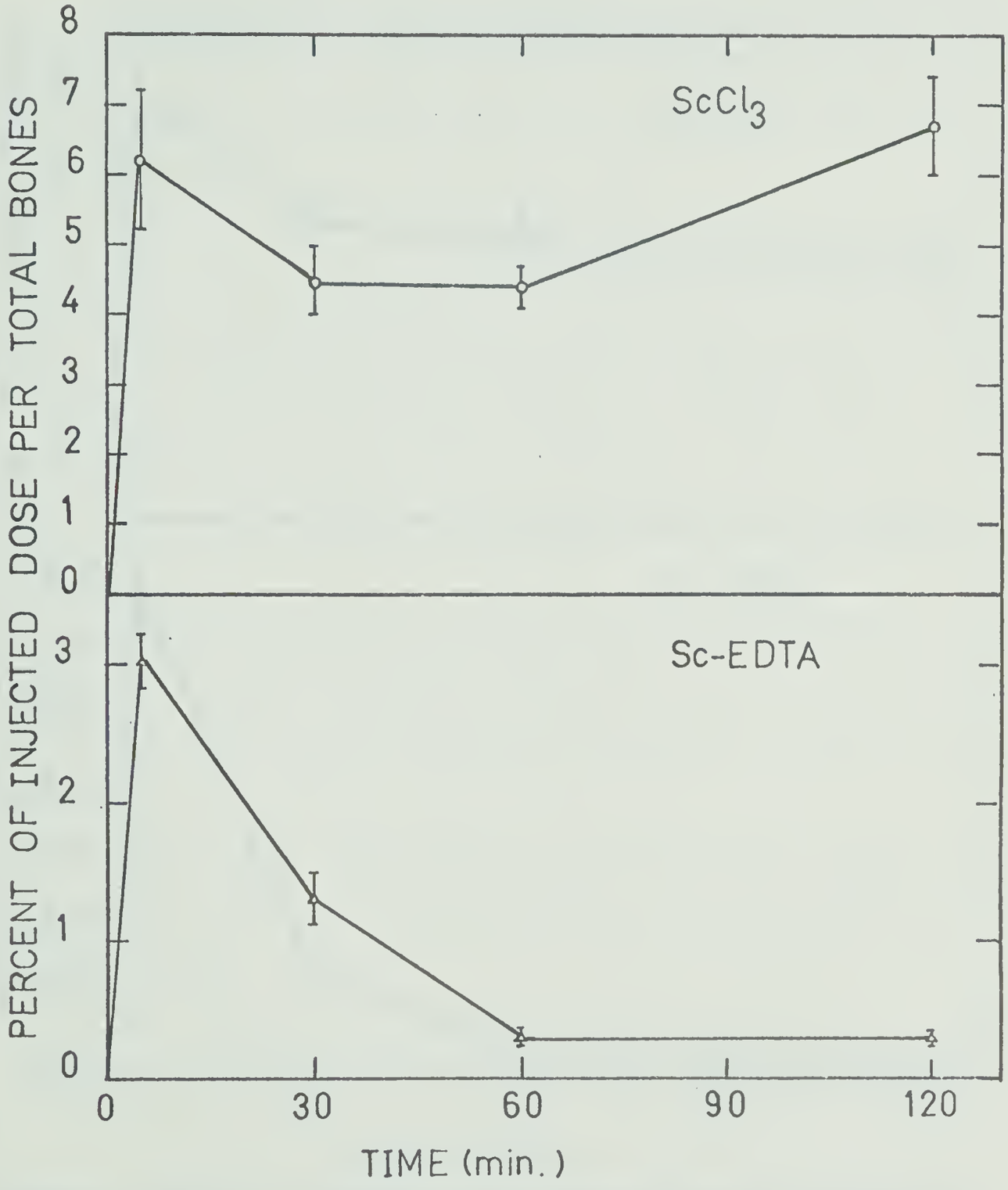


Figure 5. Uptake of scandium-46 by the mouse bone after intravenous injection

Vertical bars I represent the standard error of the mean.

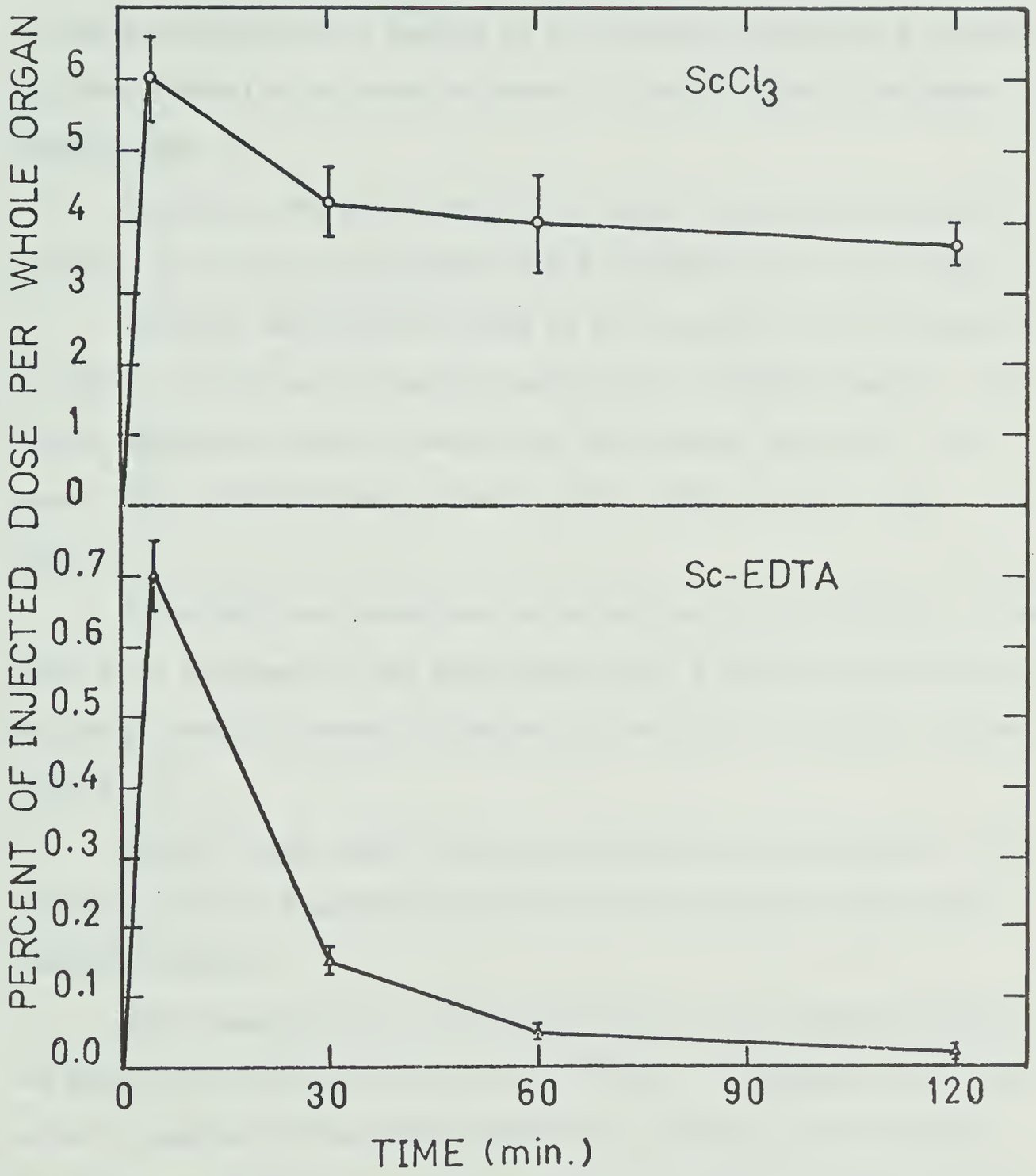


Figure 6. Uptake of scandium-46 by the mouse lungs after intravenous injection

Vertical bars I represent the standard error of the mean.

about 12 percent of the administered dose, but this level sharply declined to reach only 0.7 percent after 2 hours (Fig. 4).

The next highest uptake of ^{46}Sc-EDTA was by the kidneys, whereas they contained about 6 percent of the injected dose after 5 minutes, the level gradually decreased to about 0.7 percent after a two-hours interval (Fig. 8).

Initially, the bone concentrated about 3 percent of the injected dose, but the level decreased to 0.3 percent after 2 hours (Fig. 5).

Although the liver was shown to be the major site of deposition of $^{46}ScCl_3$, it did not concentrate much of the ^{46}Sc-EDTA complex. The percent uptake was only 1.5 percent of the injected dose after 5 minutes. The level declined gradually to 0.2 percent after 2 hours (Fig. 3).

^{46}Sc-EDTA blood level was not as high as that of $^{46}ScCl_3$, it was found to be 9 percent of the administered dose 5 minutes post-injection, declining sharply to reach 0.2 percent at the end of a 2-hours interval (Fig. 9).

Spleen, lungs, heart, pancreas and the brain were found to concentrate only very negligible quantities of the injected radio scandium-EDTA complex.

The concentration of ^{46}Sc-EDTA complex in the stomach contents was found to be different from that of $^{46}ScCl_3$. Although it was found to be 0.1 percent of the administered dose 5 minutes after injection, the level was greatly increased after one hour to 2.3 percent, and later declined to about 1.7 percent. These levels are much higher than those of $^{46}ScCl_3$.

The intestine and its contents concentrated about 1.8 percent

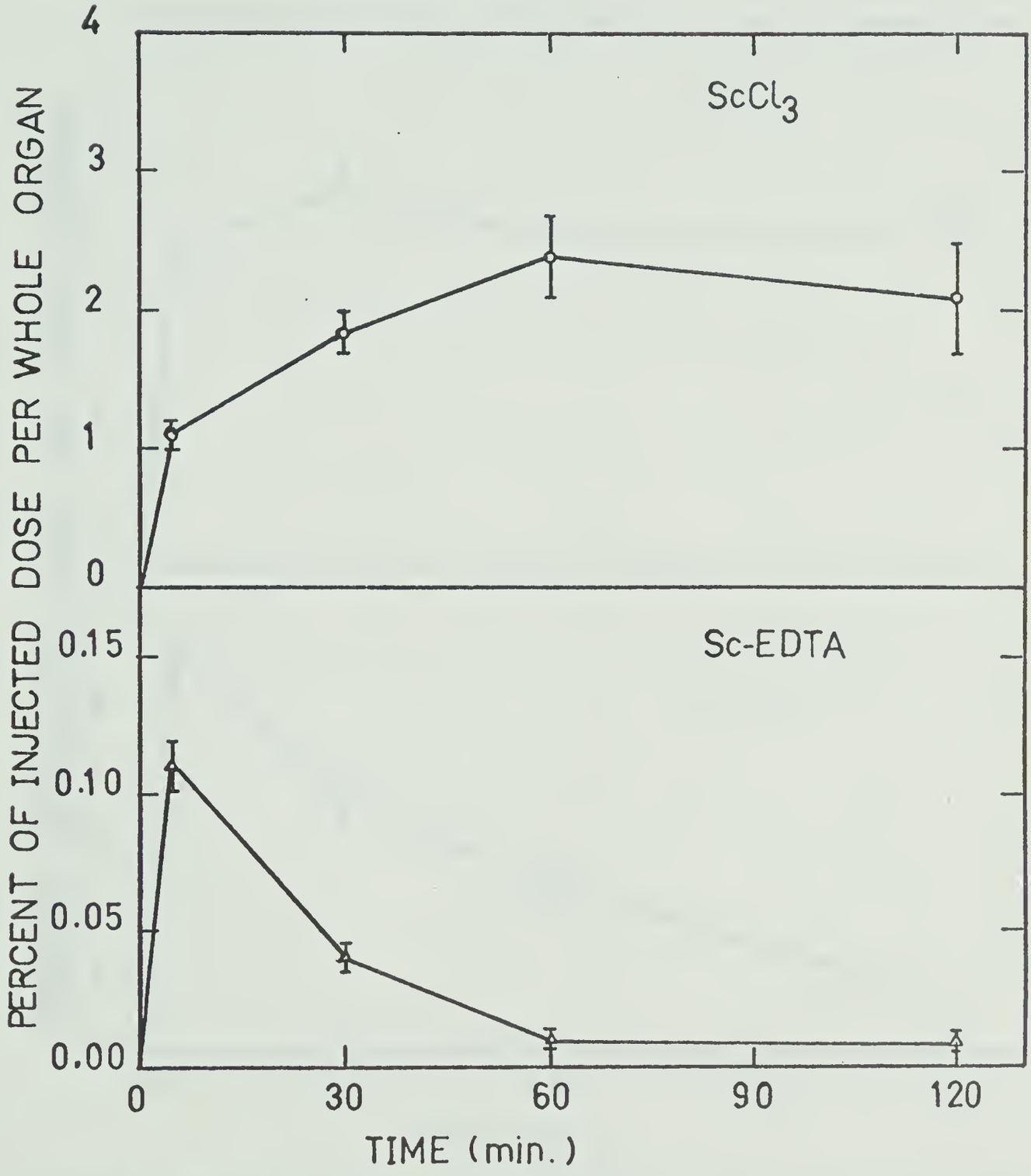


Figure 7. Uptake of scandium-46 by the mouse spleen after intravenous injection

Vertical bars I represent the standard error of the mean.

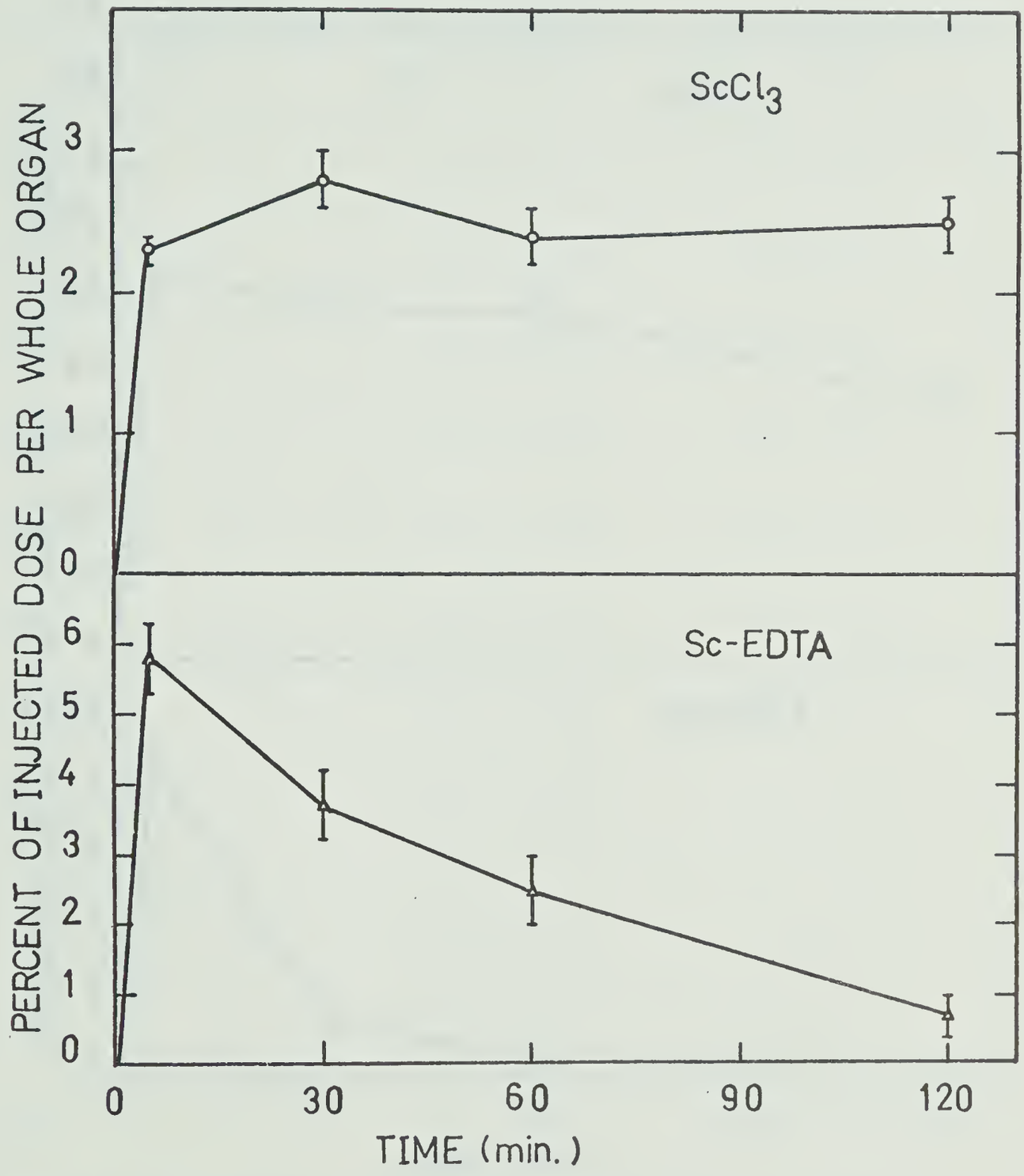


Figure 8. Uptake of scandium-46 by the mouse kidneys after intravenous injection

Vertical bars I represent the standard error of the mean.

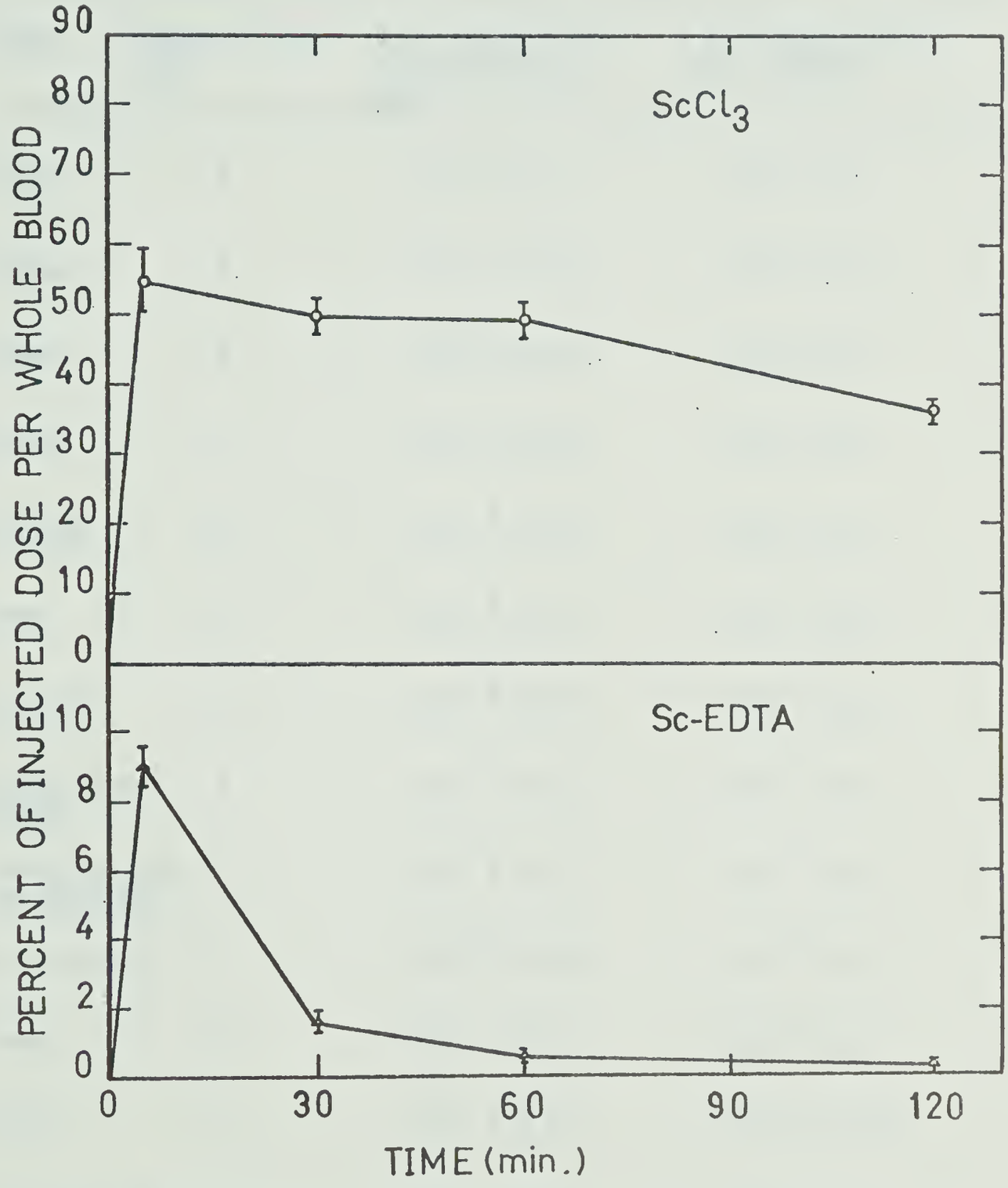


Figure 9. Mouse blood levels of scandium-46 after intravenous injection

Vertical bars I represent the standard error of the mean.

TABLE IX*

Tissue distribution of ^{46}Sc-EDTA complex five minutes after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	8	1.48 ± 0.11	0.90 ± 0.04
spleen	8	0.11 ± 0.01	1.00 ± 0.08
lungs	8	0.69 ± 0.05	3.30 ± 0.22
heart	8	0.18 ± 0.01	1.38 ± 0.07
kidneys	8	5.81 ± 0.49	9.51 ± 0.74
bone	8	2.98 ± 0.15	1.65 ± 0.08
muscles	8	12.17 ± 0.72	0.90 ± 0.05
stomach contents	8	0.10 ± 0.04	0.20 ± 0.06
intestine and ist contents	8	1.84 ± 0.13	0.73 ± 0.06
gall bladder	7	0.009 ± 0.001	3.40 ± 0.32
brain	8	0.07 ± 0.01	0.18 ± 0.02
blood	8	9.04 ± 0.68	3.93 ± 0.30[d]
urinary bladder	7	0.33 ± 0.06	2.49 ± 0.37
pancreas	7	0.21 ± 0.03	0.93 ± 0.03

* for the legend to this table see page 33

TABLE X*

Tissue distribution of ^{46}Sc-EDTA complex thirty minutes after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	6	0.53 ± 0.03	0.30 ± 0.02
spleen	6	0.04 ± 0.009	0.33 ± 0.06
lungs	6	0.15 ± 0.03	0.72 ± 0.14
heart	6	0.08 ± 0.04	0.62 ± 0.16
kidneys	12	3.72 ± 0.60	4.90 ± 0.89
bone	6	1.33 ± 0.16	0.74 ± 0.09
muscles	6	3.45 ± 0.67	0.25 ± 0.05
stomach contents	6	0.22 ± 0.05	1.11 ± 0.44
intestine and its contents	6	0.50 ± 0.15	0.23 ± 0.08
gall bladder	6	0.008 ± 0.001	2.67 ± 0.26
brain	6	0.03 ± 0.01	0.08 ± 0.03
blood	6	1.73 ± 0.22	0.75 ± 0.09[d]
urinary bladder	6	0.25 ± 0.08	2.43 ± 0.44
pancreas	7	0.03 ± 0.006	0.06 ± 0.01

* for the legend to this table see page 33

TABLE XI*

Tissue distribution of ^{46}Sc-EDTA complex one hour after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	6	0.33 ± 0.03	0.17 ± 0.01
spleen	7	0.01 ± 0.002	0.15 ± 0.04
lungs	7	0.05 ± 0.01	0.23 ± 0.03
heart	7	0.004 ± 0.001	0.04 ± 0.01
kidneys	12	2.49 ± 0.55	3.10 ± 0.52
bone	6	0.29 ± 0.05	0.14 ± 0.03
muscles	6	0.68 ± 0.06	0.05 ± 0.00
stomach contents	7	2.26 ± 0.63	15.80 ± 4.40
intestine and its contents	7	1.50 ± 0.52	0.57 ± 0.10
gall bladder	7	0.001 ± 0.000	0.33 ± 0.08
brain	7	0.05 ± 0.02	0.06 ± 0.02
blood	7	0.53 ± 0.07	0.23 ± 0.03[d]
urinary bladder	7	0.08 ± 0.02	1.97 ± 0.09
pancreas	6	0.03 ± 0.01	0.06 ± 0.02

* for the legend to this table see page 33

TABLE XII*

Tissue distribution of ^{46}Sc-EDTA complex 2 hours after injection[a,b]

Organ	Number of animals	% of injected dose per whole organ[c]	% of injected dose per g tissue[c]
liver	7	0.21 ± 0.01	0.13 ± 0.01
spleen	7	0.01 ± 0.004	0.10 ± 0.03
lungs	7	0.02 ± 0.002	0.06 ± 0.01
heart	7	0.004 ± 0.001	0.03 ± 0.01
kidneys	7	0.73 ± 0.16	0.96 ± 0.15
bone	7	0.26 ± 0.02	0.15 ± 0.01
muscles	7	0.67 ± 0.15	0.05 ± 0.01
stomach contents	7	1.56 ± 0.23	16.03 ± 3.46
intestine and its contents	7	2.66 ± 0.56	1.26 ± 0.34
gall bladder	6	0.003 ± 0.000	0.77 ± 0.15
brain	6	0.01 ± 0.00	0.02 ± 0.00
blood	7	0.19 ± 0.03	0.08 ± 0.01[d]
urinary bladder	6	0.03 ± 0.004	0.84 ± 0.10
pancreas	6	0.02 ± 0.004	0.05 ± 0.004

* for the legend to this table see page 33

of the injected ^{46}Sc-EDTA dosage after 5 minutes post-administration, decreased to 0.5 percent after 30 minutes, and reincreased to about 2.7 percent of the injected dose after 2 hours. These levels are still lower than those of $^{46}ScCl_3$, and on the other hand, very small as compared to the urinary levels.

The rapid decline in tissue levels of ^{46}Sc-EDTA complex was accompanied by an increased urinary excretion. The difference in activity between the injected dose and that concentrated in tissues can be almost detected in the urine. After two hours had elapsed, urinary excretion of ^{46}Sc-EDTA complex ranged between 90 and 95 percent of the administered dose.

D. Excretion and Biological Turnover of ^{46}Sc-EDTA Complex

Due to the rapid tissue desaturation and urinary excretion of ^{46}Sc-EDTA complex noticed in the distribution studies, whole body counting was done to determine the biological turnover of the complex. The experiments were conducted on seven animals whereby they were counted for the remaining radioactivity in the body using the method described previously. Using a semi-log paper, a graph was drawn for the remaining radioactivity as a function of time.

Using the method described by Dick and Lee (81) for analysis of any bound fraction, it was found that no bound activity could be accounted for, and the curve followed an exponential function only.

Curve peeling was accomplished using a computer program employing weighed regression and consulting t tables.

Our results revealed that tissue desaturation or excretion of ^{46}Sc-EDTA complex via the urine is an exponential function involving

three compartments;

1) A slow compartment constituting about 3.5 percent of the injected dose, and having a biological half-life of 5351 minutes.
2) A medium compartment of about 22 percent of the dose administered, and a biological half-life of 40 minutes.
3) A fast compartment constituting 75 percent of the injected dose, and having a biological half-life of 13 minutes.

Figure 10 shows the results of curve peeling of data obtained from animal number 2 as an example, whereas Table XIII shows the results of the seven animals.

Furthermore, a graph was constructed by plotting the mean of radioactivity remaining in the seven animals versus time, curve stripping was done visually. Similar results to those achieved by computer stripping were obtained with slight variations as shown in Table XIV.

Figure 10. Whole body excretion and biological turnover of ^{46}Sc-EDTA complex after intravenous injection.

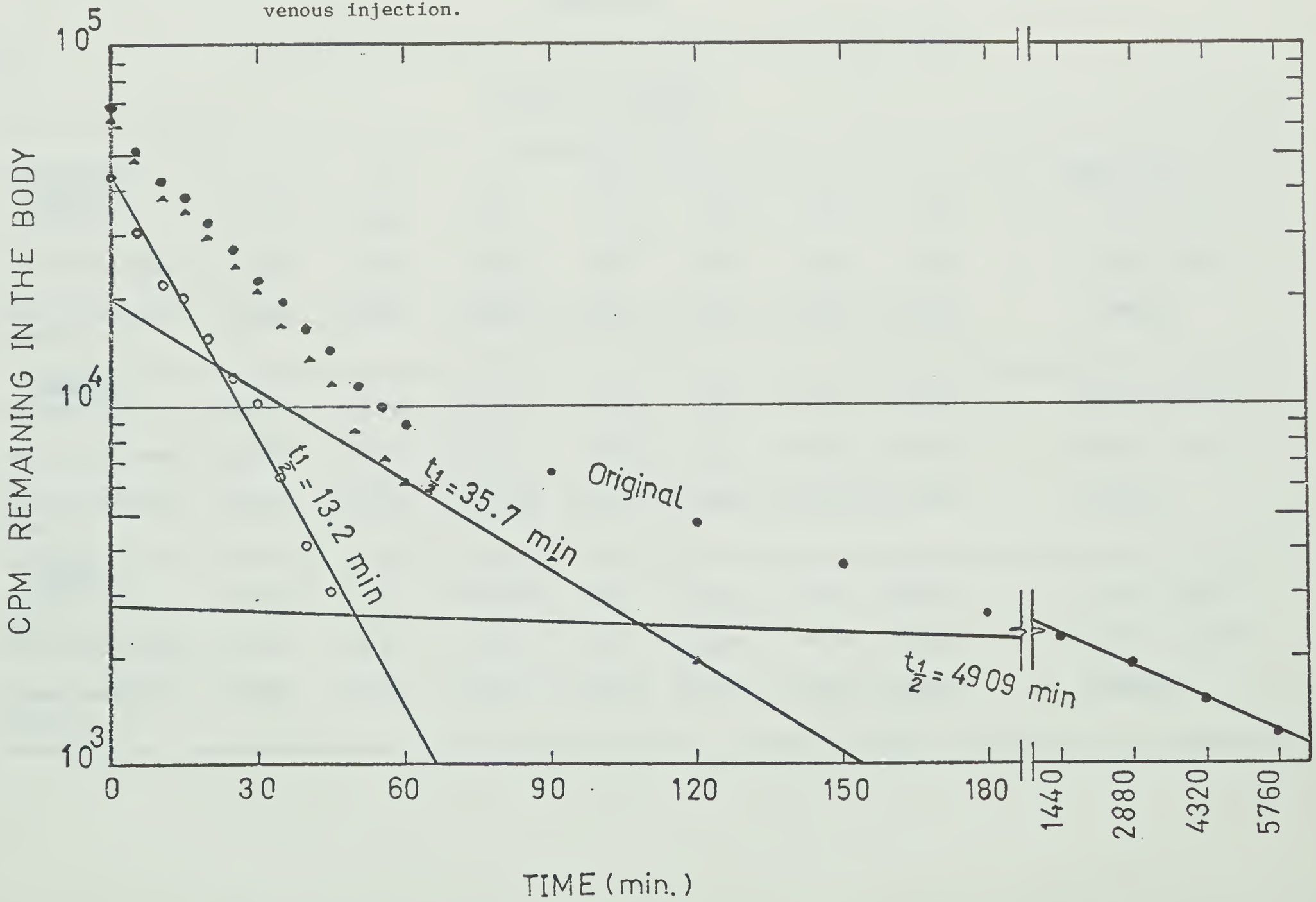


TABLE XIII

Whole body excretion and biological turnover of Sc-EDTA complex

(computer stripping)

Compartment	Experiment number 1	2	3	4	5	6	7	mean ± S.E.
slow								
volume (%)	3.73	4.22	3.45	3.56	3.52	3.37	3.35	3.60 ± 0.11
half-life (min.)	5301	4909	5394	6764	5324	4725	5042	5351 ± 253
decay constant (min^{-1})	0.00013	0.00014	0.00012	0.00010	0.00013	0.00014	0.00013	0.00013
medium								
volume (%)	23.84	29.94	19.57	23.16	19.68	19.64	16.70	21.79 ± 1.64
half-life (min.)	38.01	35.73	41.25	37.81	40.94	40.22	47.34	40.19 ± 1.41
decay constant (min^{-1})	0.01823	0.01940	0.01679	0.01832	0.01692	0.01723	0.01413	0.01722
fast								
volume (%)	74.51	63.01	79.49	75.12	73.11	75.92	81.26	74.63 ± 2.72
half-life (min.)	12.84	13.18	13.22	12.73	12.09	9.29	15.89	12.75 ± 0.73
decay constant (min^{-1})	0.05397	0.05257	0.05242	0.05443	0.05732	0.07459	0.04343	0.05540

TABLE XIV

Whole body excretion and biological turnover of ^{46}Sc-EDTA complex[a] (visual stripping)

Compartment	Volume %	Biological $t_{\frac{1}{2}}$ (minutes)	Decay constant min^{-1}
slow	3	6000	0.00012
medium	25	27	0.02567
fast	72	9	0.07700

a) Average of 7 animals

V. DISCUSSION

The determination of the LD_{50} of $ScCl_3$ and Sc-EDTA complex was effected prior to the tissue distribution studies to establish the toxic and effective levels of these compounds.

Our results revealed that the intraperitoneal LD_{50} of $ScCl_3$ in mice, with 95 percent confidence limits, is 440 (266 to 726)mg/kg. This does not coincide with the results of Haley et al. (17) who indicated that the intraperitoneal LD_{50} of $ScCl_3$ in mice was 755mg/kg. The lack of coincidence of the results could be due to either animal strain differences and/or the time of observation used by the previous investigators. While our observation time was limited to 24 hours, that used in the Haley et al., experiments was 7 days. The latter figure is not recommended for acute toxicity determinations.

The intravenous LD_{50}/24 hours of $ScCl_3$ was found to be 24 (22.6 to 25.4)mg/kg which is very small compared to the intraperitoneal LD_{50}. Thus, the intravenous route toxicity of $ScCl_3$ is far greater than that of the intraperitoneal one. The explanation of this phenomenon lies in the fact that scandium, as well as other rare earth elements, is not readily,and incompletely absorbed from parenteral routes other than the intravenous one when injected as readily ionizable salts such as the chloride (84). The case differs if rare earths are injected as weakly ionizable complexes whereby a greater absorption is encountered (49).

Using Sc-EDTA complex, the intraperitoneal LD_{50}/24 hours with 95 percent confidence limits was determined to be 720 (667 to 778)mg/kg, while the intravenous LD_{50}/24 hours was 108 (102 to 114)mg/kg.

Comparing these with the LD_{50}'s of $ScCl_3$, it is clear that the chelation or complexation of scandium decreases its toxic effects as shown by the marked increase in the LD_{50} of the complex. As the rare

earths chelates are known to be weakly ionizable, it seems that the chelating agent (EDTA in the present case) holds the scandium ion firmly enough to prevent its rapid release and production of toxic effects. This agrees with previous reports (24), on the effect of EDTA chelation on the hypotension produced by rare earths where it has been shown that EDTA chelates do not produce a marked hypotension as is the case with readily ionizable salts.

As the rare earths are known to form hydroxy radio-colloids at physiological pH when injected in tracer concentrations (42, 85), the injection of scandium as well as other rare earths in the form of chlorides, leads to their ionization releasing the metal ions which are capable of combination with hydroxyl, carbonates and phosphates ions present in the serum forming macro-molecules or colloidal aggregates (50). These colloidal aggregates or macro-molecules may be trapped by the macrophages and then distributed in the reticulo-endothelial system. They are thus largely deposited in the liver and to a lesser extent in the spleen (51).

On the other hand, since rare earths are known to bind to plasma proteins and amino acids (41, 52, 53, 54), it is conceivable that complexes of radioactive scandium and organic components are formed. Some of these macro-molecules are eventually also trapped by the reticulo-endothelial system. These observations might partially account for the high deposition of $^{46}ScCl_3$ in the liver and spleen.

Another factor contributing to the high uptake of radioactive scandium chloride, is that some rare earths are able to penetrate the liver cells and take part in their metabolism (50).

The high plasma levels of $^{46}ScCl_3$, and its slow escape from the

vascular space noticed in the present investigation might be due to colloid formation, and to the binding of scandium to serum protein fraction specially beta globulin (55). Thus, the scandium globulin complexes formed, and which do not dissociate easily, are slowly removed from plasma by the organs of the reticulo-endothelial system.

Our results revealed a relatively high uptake of $^{46}ScCl_3$ by the skeleton. This could be attributed to the possibility that some of $ScCl_3$ remained in molecular form and when passing through the capillaries it may have undergone association with either the organic matrix or the calcified areas of the bone, such behaviour has been ascribed to other rare earths (50).

Another possible explanation accounting for part of the bone uptake is that when $^{46}ScCl_3$ is injected some of it dissociates rapidly and forms macro-molecules part of which are trapped by the liver and spleen, while the rest may dissociate more slowly to particles of smaller size which are then deposited in the bone marrow (51). It has also been suggested that scandium as well as other rare earths might exchange with cations in the bone crystal itself (56), that the rare earths might associate with the organic matrix (40, 57, 58), or that they might be adsorbed onto the mineralized or the inorganic areas of the bone (59).

Our results on ^{46}Sc distribution agreed to some extent with the data reported by Rosoff *et al.* (50). The difference in results noticed lies in that the above authors used ^{46}Sc-citrate, a weak chelate.

Although the present investigation showed that the major site of deposition of $^{46}ScCl_3$ in mice is the liver yet, human data obtained after administration of ^{46}Sc-NTA, an intermediate chelate, showed that the main site of concentration was the spleen. In this case, the per-

cent uptake was higher than the liver and bone (63).

The data obtained from our investigation revealed that the main pathway of elemination of $^{46}ScCl_3$ from the body is via the gastro-intestinal tract, as the level of ^{46}Sc in the intestine and its contents was greatly higher than urinary levels. These observations coincide with the results of Rosoff et al. (50, 63), where as similar observations were reported in both mice and man.

The fecal excretion of $^{46}ScCl_3$ might be due to transfer of scandium from the liver through the bile into the intestine. Human studies supported this suggestion (63). The chemical form in which ^{46}Sc is excreted in the urine or stools is unknown.

The majority of the rare earths chlorides and readily ionizable salts were found to be excreted mainly via the intestine (41, 50, 63, 86).

Since the chelating agents are not metabolized in the body and are quantitatively excreted (87), therefore following the injection of rare earths chelates, the excretion of the rare earth will be dependant on the stability constant of its chelate. A relationship between the stability constant of the rare earth chelate and its ionic radius has been postulated (3). The smaller the ionic radius, the higher is the stability constant of the rare earth chelate, and the greater is its excretion.

Scandium, having an ionic radius of 0.68Å , which is smaller than other rare earths, is able to form a strong and a highly stable chelate with EDTA. The deposition and excretion of ^{46}Sc can be determined by the competition between the chelating agent (EDTA) and the or-

ganic and inorganic body constituents for its ion. ^{46}Sc-EDTA complex being a strong and stable chelate, it undergoes very little dissociation and will be readily excreted by the kidneys. This might explain the rapid decline in tissue and plasma levels of ^{46}Sc-EDTA complex noticed in the present investigation as the complex is rapidly excreted by the kidneys. The fairly high concentration of the complex in the stomach contents could be due to the excretion via the gastric mucosa. On the other hand, the fecal excretion was shown to be very small as compared to the urinary excretion.

The results of compartmental analysis of ^{46}Sc-EDTA turnover in the body might be explained in terms of plasma proteins, amino acids and nucleic acid binding, as they compete with the EDTA for scandium ions. The slow fraction shown in the present work might be attributed to a small degree of dissociation of ^{46}Sc-EDTA complex, whereas scandium ion is released to form colloidal aggregates which are trapped by the reticulo-endothelial system and deposited in the liver and spleen. It might also be due to association with the bone, or even adsorption onto the mineralized part of the bone. On the other hand, the intermediate compartment noticed in the present investigation could be attributed to variable degrees of binding to body proteins. This binding seems to be fairly weak as the complex is released to be cleared up by the kidneys. On the other hand, the fast compartment could be explained as mere filtration of the injected radioactive complex by the kidney to be excreted through the urine.

Further rigorous studies on the kinetics of excretion of ^{46}Sc-EDTA complex are necessary to achieve possible explanations to the behaviour of the complex in the body.

VI. SUMMARY AND CONCLUSIONS

A. The LD_{50} of $ScCl_3$ and Sc-EDTA complex in mice were determined using two routes of administration namely the intraperitoneal and the intravenous methods.

B. The I.P. and I.V. LD_{50}'s of $ScCl_3$ were found to be 440 (266 to 726) mg/kg respectively, while the I.P. and I.V. LD_{50}'s for Sc-EDTA complex were 720 (667 to 778)mg/kg and 108 (102 to 114)mg/kg respectively.

C. Various tissues of the mouse were analyzed for their contents of $^{46}ScCl_3$ and ^{46}Sc-EDTA complex following intravenous administration.

D. Distribution and excretion of scandium-46 were studied at time intervals of 5, 30, 60 and 120 minutes post-injection.

E. $^{46}ScCl_3$ was rapidly concentrated in the liver, spleen bone, muscles and lungs, and the blood levels remained high throughout the time intervals studied, while organs like the heart, brain, kidneys and stomach contents did not concentrate much of the injected dose.

F. ^{46}Sc-EDTA complex was not retained in the body tissues to any appreciable extent, and on the contrary it was rapidly and quantitatively excreted in the urine. Almost 90 to 95 percent of the injected dose was excreted within 2 hours.

G. The biological turnover of ^{46}Sc-EDTA complex was studied using whole body counting. It was found that the excretion of ^{46}Sc-EDTA complex involved three compartments; a slow compartment with a biological half-life of 5351 minutes and constituted 3.6 percent of the injected dose, a medium compartment having a biological half-life of 40 minutes and a volume of 22 percent of the dose administered, and a fast compartment with biological half-life of 13 minutes and consti-

tuted 75 percent of the injected dose.

H. Compartmental analysis of ^{46}Sc-EDTA complex showed that there was no permanently bound activity.

I. $^{46}ScCl_3$ is retained in the body mainly in the reticulo-endothelial system and the skeleton.

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